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Brief Research Communication

Gly(247) → Asp Proenkephalin A Mutation Is Rare in Schizophrenia Populations

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Schizophrenia is a complex and severe disorder of unknown cause and pathophysiology. In previous work examining an opioid hypothesis for schizophrenia, we identified a missense mutation (Gly(247) → Asp) in the proenkephalin A gene of one African-American patient. In the current study involving an extended set of African-American and other patients, we sought to identify additional mutant alleles and to determine the distribution of these alleles among several racial groups. However, the Gly(247) → Asp allele was not detected in any of 116 African-American (67 cases, 49 controls), 659 Caucasian, 1 Hispanic, 4 Asian, and 7 Native American individuals. Therefore, it appears that this mutation is a rare event of unknown clinical significance. Am. J. Med. Genet. 74:213–215, 1997.

KEY WORDS: schizophrenia; genetics; proenkephalin

INTRODUCTION

In screening schizophrenic patients for sequence variations within the proenkephalin gene, we detected one missense mutation of possible functional significance [Mikesell et al., 1996]. This Gly(247) → Asp mutation occurred within a highly conserved enkephalin-containing peptide and changed a nonpolar to a charged amino acid between two nearby enkephalin peptides, suggesting that processing/cleavage events could be affected. However, because this mutation was detected only in 1 of 46 African-American patients (but not in a comparable number of African-American controls), its association with schizophrenia was unclear.

Family members of the positive individual were not available for cosegregation analysis. Thus, we undertook an extended search for the mutation in additional African-American and other patients. To rapidly screen for the mutant allele, we developed a polymerase chain reaction (PCR)-based restriction fragment length polymorphism assay. The mutation creates a recognition site for the restriction enzyme *Bbs*I, and no other sites for this enzyme are present in the 1,100-bp PCR product of exon III of proenkephalin. Therefore, PCR of exon III followed by restriction digestion and agarose gel electrophoresis yields two bands (650 and 450 bp) for positive alleles, while leaving the 1,100-bp product intact for negative alleles (Fig. 1A).

We used this assay to screen 671 non-African-American and 116 African-American individuals for the Gly(247) → Asp mutation. The non-African-American samples were obtained from a large collection of cases and controls described by Sobell et al. [1993]. All schizophrenic cases were diagnosed by a research psychiatrist (L. Heston, M.D.) based on DSM-III-R criteria [American Psychiatric Association, 1987]. Strict fulfillment of the diagnostic criteria was assessed primarily through review of medical records. The overwhelming majority of patients were chronic schizophrenics who were ascertained as research volunteers while in state hospitals. Lengthy, detailed medical records, including archived records, were available for most patients [Sobell et al., 1993]. Patients screened were: 512 Caucasians of Northern or Western European descent, 3 Asians, and 7 Native Americans. Also screened were the following psychotic individuals not strictly fulfilling the diagnostic criteria for schizophrenia: 147 Caucasians, 1 Hispanic, and 1 Asian. DNA was extracted from peripheral blood as previously described [Gustafson et al., 1987].

The African-American subjects included 67 cases (37 male) as well as 49 controls (19 male). The cases were inpatients and outpatients who fulfilled DSM-III-R

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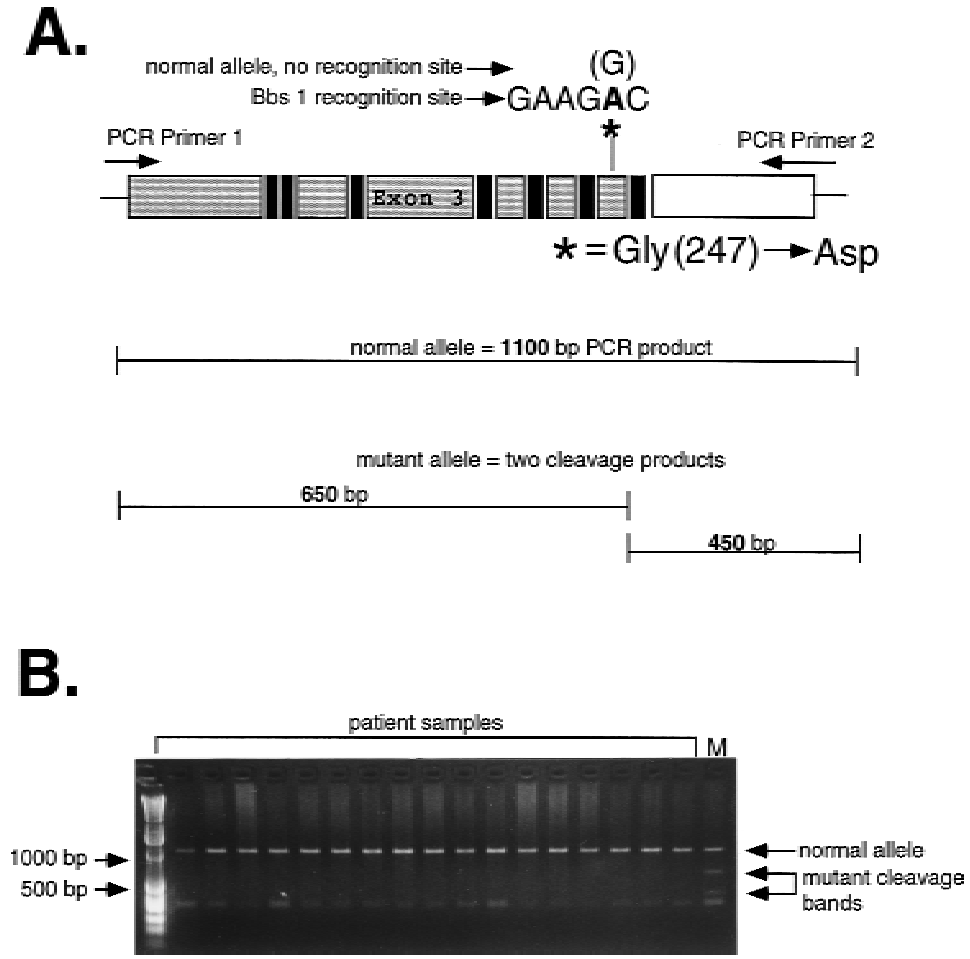

Fig. 1. Restriction fragment length polymorphism assay for Gly(247) → Asp mutation. **A:** Schematic representation of the assay. In the depiction of the third exon of the human proenkephalin gene, the exon is indicated as a box; introns are represented as lines (not to scale). Shaded area represents the translated region. Black boxes are enkephalin peptides. PCR was performed using downstream and upstream primers 1 and 2 as shown, corresponding to primers 4D and 9U in Mikesell *et al.* [1996]. The 20-μl reactions contained 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH 8.8, 0.1% Triton X-100, 8 mM $MgSO_4$, 0.1 μM each primer, 0.2 mM each dNTP, approximately 0.1 μg genomic DNA, and 1 U Deep-Vent DNA Polymerase (exo–) (New England Biolabs, Beverly, MA). Cycling conditions were: 98°C for 3 min, followed by 30 cycles of 98°C for 1 min, 44°C for 5 sec, and 75°C for 1 min, with a final extension at 75°C for 10 min. The expected full-length exon 3 PCR product of 1,100 bp is shown schematically, as well as the two predicted restriction fragments generated from the *Bbs*I recognition site GAAGAC created by the mutation. **B:** Representative agarose gel photograph. Following PCR amplification, products were incubated with 2 units of *Bbs*I (New England Biolabs) for 2 hr at 37°C. Loading buffer (80% glycerol, 0.1% each of xylene cyanol and bromophenol blue dyes) was added and reactions were separated on 2% agarose gels at 80 V for 1.5 hr. Gels contained 10 μg ethidium bromide per 100 ml solution. A 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD) is shown in the first lane, with relevant sizes marked on the left. The final lane (**M**) contains the positive control (original patient with the mutation), in which the restriction fragments of predicted length can be seen. All other lanes contain patient samples, none of which show the restriction fragments indicating the presence of the mutation. A spurious amplification product of approximately 400 bp is evident in each lane.

(and DSM-IV) criteria for schizophrenia. They were recruited from the Western Psychiatric Institute and Clinic (WPIC), Pittsburgh, a tertiary care facility which also serves as a catchment area hospital for a defined region of Allegheny Country, Pennsylvania. Clinical information about the cases was obtained from case notes as well as from a semistructured interview. This information was recorded using the Operational Criteria (OPCRIT) checklist [McGuffin et al., 1991]. The controls were adults screened for the absence of a history (past or current) suggestive of psychotic illness or alcohol/drug abuse.

Each sample was amplified by PCR as described in Figure 1. PCR products were then treated with the *Bbs*I restriction enzyme, and the fragments were separated by agarose gel electrophoresis. Bands were detected by ethidium bromide staining. An advantage of this method was that we could confirm amplification of every sample, as negative samples will still show a full-length PCR product. Figure 1B shows a representative gel result. Positive samples which were heterozygotes showed the full-length PCR product plus an additional two bands (Fig. 1B, lane M). No additional mutant alleles were detected in any of the nearly 800 individuals screened.

We computed exact two-sided 95% confidence intervals for binomial proportions to estimate the possible prevalence of the mutation using Splus Statistical Software (Mathsoft, Inc. (Seattle, WA)). Included in the calculation were individuals screened in this study and the 46 African-American patients and 40 African-American controls analyzed in previous work [Mikesell

et al., 1996]. Since we observed only a single incidence of the mutation, we estimated the range of prevalence using two extreme models. If the mutation is neither disease- nor race-associated, it is valid to combine all individuals screened. The observed mutation occurred in 1 of 873 individuals. For 1/873, the prevalence of the mutation was found to be between 0.003–0.64% with 95% confidence. If, on the other hand, the mutation is associated with both race and disease, the observed mutation occurred in 1 of 113 African-American schizophrenic patients. In this case, the prevalence of the mutation was found to be between 0.02–4.38% with 95% confidence. Using any model, we are 95% confident that the true prevalence of this mutation is not greater that 4.4% and not less than 0.003%. We conclude that this mutation is a rare event. However, determination of its clinical significance must await further study.

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